A comparison of the metabolism of eighteen-carbon ^{13}C-unsaturated fatty acids in healthy women

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Abstract Altered use of different dietary fatty acids may contribute to several chronic diseases, including obesity, noninsulin-dependent diabetes mellitus, and cardiovascular disease. However, few comparative data are available to support this link, so the goal of the present study was to compare the metabolism of [^{13}C]oleate, [^{13}C]α-linolenate, [^{13}C]elaidate, and [^{13}C]linoleate through oxidation and incorporation into plasma lipid fractions and adipose tissue. Each tracer was given as a single oral bolus to six healthy women. Samples were collected over 8 days, and ^{13}C was analyzed using isotope ratio mass spectrometry. At 9 h postdose, cumulative oxidation was similar for [^{13}C]elaidate, [^{13}C]oleate, and [^{13}C]α-linolenate (19 ± 1%, 20 ± 4%, and 19 ± 3% dose, respectively). Significantly lower oxidation of [^{13}C]linoleate (12 ± 4% dose; $P < 0.05$) was accompanied by its higher incorporation into plasma phospholipids and cholesteryl esters. Abdominal adipose tissue was enriched with [^{13}C]α-linolenate, [^{13}C]elaidate, or [^{13}C]linoleate within 6 h. The percentage linoleate in plasma phospholipids correlated positively with [^{13}C]linoleate and [^{13}C]elaidate oxidation, indicating a potential role of background diet. Conversion of [^{13}C]linoleate and [^{13}C]α-linolenate to longer chain polyunsaturates was a quantitatively minor route of utilization.—McCloy, U., M. A. Ryan, P. B. Pencharz, R. J. Ross, and S. C. Cunnane. **A comparison of the metabolism of eighteen-carbon ^{13}C-unsaturated fatty acids in healthy women. *J. Lipid Res.* 2004.** 45: **474–485.**

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Linoleate (18:2n-6) and oleate (*cis*-18:1n-9) are the two predominant unsaturated fatty acids in the diet and are associated with several health benefits (1). α-Linolenate (*cis*-18:3n-3) is much less common in the diet but also has well-known health benefits, particularly against coronary artery disease and mortality (2–5). Elaidate, the main *trans*-isomer of oleate (*trans*-18:1n−9), which is mainly formed by food industry hydrogenation of vegetable oils, is associated with health concerns, particularly an increased risk of hypercholesterolemia (6–8). Despite their different abundances in the diet and different health implications, structurally, these four fatty acids are broadly similar in having 18 carbons and one to three double bonds. Nevertheless, comprehensive, comparative information is lacking about their metabolism that could help account for their differing health attributes in humans. This could be especially helpful in identifying potential reasons why *trans*-fatty acids may be hypercholesterolemic.

The overall objective of this study was to gain insight into the differential health effects of these four common 18-carbon dietary unsaturated fatty acids by tracing their metabolism in healthy women using uniformly carbon-13 labeled (U-^{13}C) stable isotope tracers. This was a crossover study in which free-living healthy subjects consuming a self-selected diet orally ingested a tracer dose of [U-^{13}C]oleate, [U-^{13}C]elaidate, [U-^{13}C]linoleate, and [U-^{13}C]α-linolenate in random sequential order with washout periods between each tracer. ^{13}C was measured in breath (β-oxidation), plasma fatty acids, and adipose tissue (storage) by high-precision gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Thus, the amount of each tracer used for energy, esterified to different lipid classes in plasma, desaturated and chain-elongated to long-chain PUFAs or stored in adipose tissue, was measured over a period of 8 days. MRI was used to quantify adipose tissue volumes to obtain pool size. Relationships between abdominal subcutaneous fat volume, blood lipids, plasma fatty acid profiles, and the metabolism of these tracers were also determined to identify con-

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Abbreviations: AP, atom percent; APE, atom percent excess; AUC, area under the curve; CE, cholesteryl ester; GC-C-IRMS, gas chromatography-combustion-isotope ratio mass spectrometry; PL, phospholipid; U-^{13}C, uniformly carbon-13 labeled.

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tributions of lifestyle, body composition, and background diet (dietary fatty acids).

MATERIALS AND METHODS

Subjects

Six healthy, normolipidemic, premenopausal women participated in the study (**Table 1**). Subjects were excluded if they had any known endocrine conditions, a body mass index outside the healthy range of 20–27, or if they smoked. One subject was taking oral contraceptives, and another was taking milder hormonal therapy for acne control. Five of the six subjects had regular menstrual cycles (26–30 days) so were timed to be in the same phase of the cycle for all tracers. Approval for the study was obtained from the Research Ethics Board at the Hospital for Sick Children in Toronto. Written informed consent was obtained from all subjects, and remuneration was provided.

Experimental design

Baseline blood, breath, and adipose tissue samples were collected at 8 AM after a minimum 12 h fast and before tracer dosing. Subjects were then sequentially administered an oral bolus of [U-^{13}C]oleate (Isotec, Miamisburg, OH), [U-^{13}C]linoleate, [U-^{13}C]α-linolenate (both from Martek Biosciences, Columbia, MD), or [U-^{13}C]elaidate incorporated into a breakfast meal in random order with a 3 week washout between each. The [U-^{13}C]elaidate was originally purchased as [U-^{13}C]oleic acid from Isotec and then isomerized by Dr. N. Ratnayake (Health Canada, Ottawa) according to the procedure of Snyder and Scholfield (9). With the exception of [^{13}C]elaidate, all tracers were given as the free fatty acid, which was placed directly on a bagel containing a cream cheese spread via a 100 μl syringe (Hamilton, Reno, NV). [^{13}C]Elaidate was administered as the methyl ester so that it would be liquid at room temperature. The bagel and cream cheese (with tracer) were part of a breakfast meal that included fruit juice and a banana. The macronutrient composition of the breakfast was 17% fat, 9% protein, and 74% carbohydrate. On the first day of each study, identical, standardized, weighed, regularly timed meals were provided for each subject and each tracer study. The meals were consumed at 0 h (test meal) and 4, 7, and 10 h postdose. The energy value of the meals given at these time points was 525, 653, 229, and 869 kcal, respectively. Starting the weekend before the study, subjects were given a list of foods that are naturally enriched in ^{13}C, especially corn-based products, that were to be avoided during the study period (10). The effect of the natural ^{13}C content in the meals provided on background breath $^{13}CO_2$ excretion was measured on 2 separate days in one subject, and these background values were subtracted from the dosed values in all subjects.

On the first study day, 5 ml of blood was collected at 2 h intervals through a heated intravenous hand vein line flushed with saline and heparin, drawn into 5 ml syringes, and immediately transferred to EDTA-coated tubes (Becton Dickinson, Franklin Lakes, NJ) and placed on ice. Plasma was separated from erythrocytes by centrifuging at 2,500 rpm at 4°C for 10 min and stored for no longer than 2 weeks at −20°C before analysis. Breath samples were collected hourly, in duplicate, directly into evacuated glass tubes using a breath collection device (EasySampler™; Quintron Instrument Co., Milwaukee, WI). The rate of CO_2 production was determined from a variable-flow indirect calorimeter (V_{max} = 29n; Sensormedics Corp., Yorba Linda, CA). This was performed 90 min after breakfast in the postprandial period rather than at fasting because of the mainly postprandial sampling points throughout the study day. Fasting samples of blood and breath were also collected at 24, 48, 72, and 168 h postdose. Samples of subcutaneous fat (5–20 mg) were taken at 6, 24, and 168 h postdose from the anterior abdominal wall near the umbilicus using gentle suction with an 18 gauge needle and a 5 ml syringe (11) and transferred to a glass test tube filled with hexane until analyzed.

TABLE 1. Characteristics of study subjects

Age (years)	28.7 ± 4.9 (22–36)
Weight (kg)	65.0 ± 6.3 (54–71)
Height (cm)	164 ± 3.8 (159–168)
Body mass index[a]	24.1 ± 2.3 (20.5–27)
Plasma triglyceride (mmol/l)[b]	0.6 ± 0.3 (0.4–1.1)
Plasma total cholesterol (mmol/l)[c]	4.5 ± 0.6 (3.8–5.2)
Percentage body fat[d]	25.1 ± 2.5 (20.7–28.2)

Data shown are means ± SD, with ranges in parentheses.
[a] Body mass index = weight (kg)/height (m^2).
[b] Plasma triglyceride values are means of all fasting samples.
[c] Plasma cholesterol values are average of fasting blood samples taken in the first week of the study.
[d] Determined from magnetic resonance images, assuming adipose tissue is 82% water (11).

Tracer protocol

The chemical purity (measured in our laboratory) of the tracers was 93.9% for [U-^{13}C]α-linolenate, 95.2% for [U-^{13}C]linoleate, 94.4% for [U-^{13}C]oleate, and 74% for [U-^{13}C]elaidate. Each tracer had an isotopic purity of greater than 99%. The volume administered (55 μl) corresponded to 3.06 mmol of ^{13}C for α-linolenate, 2.91 mmol of ^{13}C for oleate, 2.99 mmol of ^{13}C for linoleate, and 2.73 mmol of ^{13}C for elaidate (0.69–0.91 mg/kg body weight). These values include all ^{13}C, including the contaminating fatty acids, and were used in the breath CO_2 calculations. However, for plasma and adipose tissue calculations from the GC-C-IRMS data, the actual mass of the ^{13}C fatty acids of interest was used, excluding any contaminating fatty acids. This corresponded to 33.7 mg of elaidate, 47.0 mg of α-linolenate, 47.0 mg of linoleate, and 46.7 mg of oleate. The test meal contained 2,665 mg of oleate, 152 mg of α-linolenate, 860 mg of linoleate, and 27 mg of elaidate, resulting in tracer-tracee ratios of 0.018, 0.315, 0.055, and 1.25, respectively.

Analytical methods

Plasma total cholesterol was determined using a colorimetric assay (Diagnostic Chemicals Ltd., Charlottetown, Prince Edward Island, Canada). Plasma triglyceride (TG) concentration was determined from GC analysis of plasma TG fatty acids using triheptadecanoic acid as the internal standard (Sigma Chemical Co., St. Louis, MO). Fatty acid composition of the freeze-dried test meal was determined in quadruplicate portions of the bagel and cream cheese (12). Plasma total lipids were extracted using a modified Folch procedure (13) with internal standards added before thin-layer chromatography to separate the plasma lipid classes. Plasma phospholipid (PL) and cholesteryl ester (CE) were saponified separately, and all fatty acids were methylated with BF_3 in methanol. Fatty acid methyl esters were analyzed using a gas chromatograph (model 5890A; Hewlett Packard, Palo Alto, CA) equipped with a fused capillary column (30 m × 0.25 mm × 0.25 μm; DB-23; J&W Scientific, Folsom, CA) as described previously (14). The identity of individual fatty acids was determined by comparing retention times with standard mixtures of fatty acids [NuChek 68A and NuChek 96 (NuChek Prep, Inc., Elysian, MN) and Supelco PUFA2 (Sigma-Aldrich Canada Ltd., Mississauga, Ontario, Canada)]. The concentration of ^{13}C-labeled fatty acid in the plasma lipid classes was calculated by multiplying

the percentage of ^{13}C enrichment above baseline [atom percent excess (APE)] by the concentration of dosed fatty acid.

Adequate separation of *trans*-isomers was obtained by GC (model 6890; Hewlett Packard) using a fused capillary column (100 m × 0.25 mm × 0.2 μm; SP2560; Supelco, Inc., Bellafonte, PA) as described by Ratnayake and Chen (15). Samples were run at a constant flow with an injection split ratio of 10:1. Isotopic analysis was performed by GC-C-IRMS with 100% CO_2 as the reference gas. The gas chromatograph (model 6890; Hewlett Packard) was connected to a 800°C combustion interface (Orchid GC Interface Module; PDZ Europa Ltd., Crewe, UK) that was linked to the mass spectrometer (20-20 Stable Isotope Analyser; PDZ Europa Ltd.). Isotope enrichment linearity and precision were determined by analyzing serial dilutions of [1-^{13}C]palmitate (16:0) combined with increasing amounts of palmitate at natural ^{13}C abundance. Fatty acid enrichment, with the exception of samples enriched with [^{13}C]elaidate, was analyzed with a fused capillary 30 m × 0.32 mm × 0.32 μm DB-23 column (J&W Scientific) with an injection split ratio of 5:1. Injection and detector temperatures were 230°C and 240°C, respectively. Starting temperature was 135°C for 2 min, ramped at 4°C/min to 160°C for 5 min, and then at 6°C/min with 195°C the final temperature. ^{13}C enrichment values were computed by the Orchid software (GC Post Processor version 2.3c; Europa Scientific, Crewe, UK) in units of atom percent (AP):

$$AP = \left(\frac{[^{13}C]}{[^{13}C] + [^{12}C]}\right) \times 100$$

APE was calculated by subtracting baseline AP from dosed values at each time point (t): $APE = AP_t - AP_{baseline}$. The percentage of dose per liter of plasma was calculated using the tracer concentration at each time point and the tracer dose:

$$\%\ \text{dose/l plasma} = \frac{\mu g\ ^{13}C\ \text{fatty acid/l plasma} \times 100\%}{\text{dose administered}(\mu g\ ^{13}C\ \text{fatty acid})}$$

The percentage of dose per liter of plasma was calculated for each time point and was plotted against time (h). The total area under the curve (AUC) was calculated using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA) to give a relative measure of the amount of label appearing over the 168 h period of the study. The time to reach the maximum percentage of dose per liter for each individual subject in each lipid fraction was also averaged to give a measure of the peak enrichment.

For the [^{13}C]elaidate-enriched samples, a *cis/trans*-capillary GC column was used (HP 23; 60 m × 0.32 mm × 0.25 μm) with an injection split ratio of 5:1. Injection and detector temperatures were 240°C and 250°C, respectively. The temperature program started at 170°C for 2 min, then increased at a rate of 4°C/min to 230°C, where it was held for 5 min. The chromatograms (for all but the plasma PL) had just one *trans*-fatty acid shoulder, which was assumed to be the total of 6-11t-18:1, with 12-14t-18:1 coeluting with oleate. These assumptions were based on the chromatograms using the 100 m column, which showed a near baseline separation of 11t-18:1 from 12t-18:1. Plasma PL, unlike the other plasma lipids and adipose tissue, had two *trans*-fatty acid shoulders on the main *trans*-fatty acid isomer peak, attributable to the presence of 10t-18:1 as a small portion of the total *trans*-18:1 isomers in plasma PL. Comparison of the GC and the GC-C-IRMS results indicated that the first peak contained 6-9t-18:1 and the second contained 10t-18:1 and 11t-18:1. A correction was then made to accommodate the dilution of enrichment from the coeluting peaks by GC-C-IRMS. The calculated APE of [^{13}C]elaidate was obtained by dividing the APE of the total *trans* peak (obtained from GC-C-IRMS) by the percentage of elaidate of all the coeluting *trans*-isomers (8t- + 9t- + 10t- + 11t-18:1; obtained from the *trans*-fatty acid analysis done by GC). Because only 8t- and 9t-18:1 coeluted in plasma PL, the fraction used was the proportion of 9t-/8t- + 9t-18:1:

$$APE_{9t\text{-}18:1} = \frac{APE_{total}}{\left(\frac{\%\ 9t\text{-}18:1}{\%\ 8t\text{-} + 9t\text{-} + 10t\text{-} + 11t\text{-}18:1}\right)}$$

The concentration of [^{13}C]elaidate in individual plasma lipid classes was then calculated as for the other tracers using this calculated APE value of elaidate.

The enrichment of ^{13}C in breath CO_2 was analyzed by continuous-flow IRMS with 5% CO_2 as the reference gas (20-20 Stable Isotope Analyser, PDZ Europa Ltd.). Samples were collected into evacuated tubes (Labco Ltd., Buckinghamshire, UK). Software (Ancant System version 1.999s) calculated the AP relative to the previously calibrated 5% CO_2. The linearity and precision of isotope enrichment were assessed using serial dilutions and combustion of [^{13}C]glycine. Precision was routinely very good at 0.04. Results are expressed as a percentage of the administered dose expired per hour. Cumulative oxidation was calculated from the AUC of the percentage of dose per hour versus time, as was done for plasma (GraphPad Prism version 3.0):

$$\%\ \text{dose/h} = \left(\frac{\text{APE} \times \text{mmol total } CO_2\text{expired/h}}{\text{mmol } ^{13}C\text{ administered}}\right) \times 100\%$$

where mmol CO_2 = 22.4 mol/l × l CO_2 expired/h (indirect calorimetry) and mmol ^{13}C administered = molecular weight of fatty acid × milligrams of fatty acid administered × number of ^{13}C-labeled carbons.

Magnetic resonance imaging

To obtain total and regional body fat and lean tissue composition, whole body MRI scans were taken with a General Electric 1.5 tesla whole-body scanner. From the feet to the fingertips, 41 images were taken while the subjects lay in a supine position (16). Ten millimeter thick images were acquired every 40 mm to obtain total, subcutaneous, visceral, and abdominal subcutaneous adipose tissue as well as lean tissue and skeletal muscle volumes. Changes in body weight that occurred during the delay between the MRI and the tracer studies (0–18 months, −3.8 to +2.8 kg) were corrected for by assuming that 80% of weight gain or loss was from adipose tissue, with an even distribution over the whole body. Tracer enrichment in adipose tissue was calculated as:

$$\%\ \text{dose present in ASAT} = \frac{\text{APE}/100 \times \%\text{FA} \times \text{ASAT (l)} \times 0.724/10^6}{\text{mg } ^{13}C\text{ fatty acid administered}}$$

where the percentage of fatty acid (FA) was determined by GC, the volume of abdominal subcutaneous adipose tissue (ASAT) was determined by MRI, and 0.724 kg/l is the fatty acid density of adipose tissue (11).

Plasma tracer kinetics

The disappearance of ^{13}C-labeled fatty acids from plasma was fitted to a one-phase exponential decay curve using a nonlinear regression formula (GraphPad Prism version 3.0). The rate of disappearance was the rate constant, *K*, and the half-life (h) of the decay was 0.6932/*K*. The time to peak enrichment was taken from the maximum calculated percentage of dose per liter for each individual subject in each tracer pool.

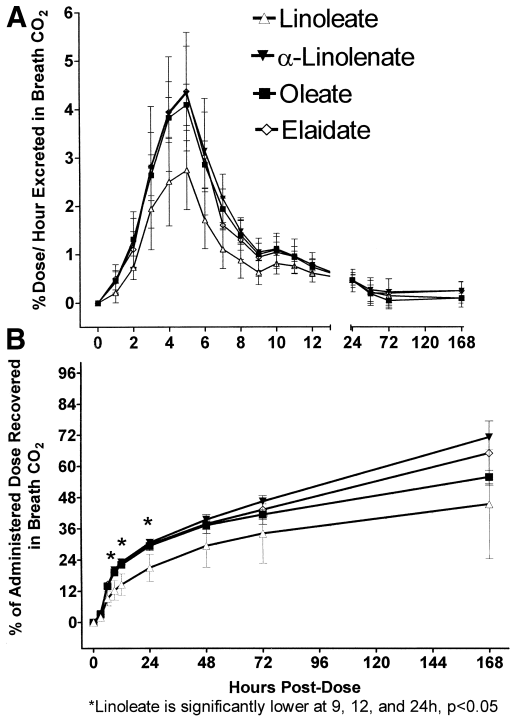

Fig. 1. Time course of ^{13}C enrichment in breath CO_2 after an oral dose of uniformly carbon-13 labeled (U-^{13}C) linoleate, oleate, α-linolenate, and elaidate given to six healthy women. A: Percentage of the administered dose recovered hourly in breath CO_2. B: Cumulative oxidation determined from area under the curve calculations from the hourly percentage of oxidation. Data points indicate means ± SD.

Statistics

All statistical analyses were performed using the software package SigmaStat (version 2.0; Jandell Scientific, San Rafael, CA). To determine differences in tracer oxidation, one-way repeated-measures ANOVA was performed, and Tukey's test was used as the post hoc test. Plasma fatty acid and adipose tissue enrichment were not normally distributed; therefore, all other comparisons were analyzed using the nonparametric Friedman repeated-measures ANOVA on ranks with Student-Newman-Keuls as the post hoc test. All correlations were performed using the nonparametric Spearman rank order correlation test.

RESULTS

Tracer oxidation

^{13}C fatty acid oxidation peaked at ~5 h postdose for all tracers (**Fig. 1**). The peak rate of oxidation was 4.9% dose/h for [^{13}C]elaidate, 4.4% dose/h for [^{13}C]oleate, 3.0% dose/h for [^{13}C]linoleate, and 4.5% dose/h for [^{13}C]α-linolenate, with [^{13}C]linoleate values being significantly lower than those of the other three tracers ($P < 0.05$; Fig. 1A). Detectable ^{13}C enrichment was present in breath CO_2 at 168 h in 22 of the 24 individual tracer studies. Cumulative fatty acid oxidation determined from AUC results was significantly lower for [^{13}C]linoleate than for [^{13}C]α-linolenate, [^{13}C]elaidate, or [^{13}C]oleate at 9, 12, and 24 h ($P < 0.05$) but not after that (Fig. 1B). The percentage of linoleate in plasma PL fatty acids (**Table 2**) correlated positively with the cumulative oxidation of both [^{13}C]elaidate and [^{13}C]linoleate at 9 h postdose ($r = 0.94$, $P = 0.02$ for both) and also at 12 and 24 h postdose for linoleate. The percentage of α-linolenate in plasma PL fatty acids correlated with the cumulative oxidation of [^{13}C]elaidate at 12 and 24 h postdose ($r = 0.89$, $P = 0.03$).

Tracer in plasma lipids

The time course of ^{13}C enrichment in plasma lipids is expressed as the percentage of dose per liter of plasma (**Fig. 2**). AUC values over the 168 h study period are listed in **Table 3**. At the time of peak enrichment, the order of enrichment in plasma TG was [^{13}C]oleate = [^{13}C]elaidate > [^{13}C]linoleate = [^{13}C]α-linolenate ($P < 0.05$). However, the AUC values in plasma TG differed somewhat and were in the order of [^{13}C]elaidate > [^{13}C]oleate > [^{13}C]linoleate > [^{13}C]α-linolenate (Table 3). From the

TABLE 2. Percentage composition of fatty acids in plasma lipid fractions and adipose tissue

Fatty Acid[a]	PL	TG	CE	Free Fatty Acid	Adipose Tissue
Σ saturates	39.77 ± 4.31	27.68 ± 3.98	13.62 ± 2.33	35.85 ± 4.69	25.16 ± 1.59
18:1n-9*cis*	10.41 ± 0.56	36.78 ± 2.97	18.48 ± 1.73	38.21 ± 2.54	41.69 ± 1.37
Σ monounsaturated fatty acid	13.39 ± 0.60	44.27 ± 3.56	22.77 ± 2.70	44.40 ± 2.36	49.49 ± 1.53
18:1n-9*trans*[b]	0.42 ± 0.21	0.74 ± 0.27	0.049 ± 0.02	ND	1.11 ± 0.29
Σ 18:1*trans*[b]	2.18 ± 1.12	2.55 ± 0.92	ND	ND	4.26 ± 1.48
18:2n-6	24.31 ± 5.21	18.33 ± 3.17	52.89 ± 6.01	13.33 ± 0.90	15.43 ± 1.80
20:3n-6	3.37 ± 0.62	0.30 ± 0.03	0.79 ± 0.15	0.17 ± 0.03	0.17 ± 0.09
20:4n-6	10.34 ± 2.25	1.13 ± 0.31	6.44 ± 1.48	0.61 ± 0.17	0.33 ± 0.06
Σ n-6 PUFA	39.33 ± 4.98	20.31 ± 3.20	60.98 ± 5.31	14.69 ± 0.97	16.25 ± 1.96
18:3n-3	0.42 ± 0.25	1.64 ± 0.53	0.96 ± 0.43	1.21 ± 0.15	1.45 ± 0.33
20:5n-3	1.51 ± 1.19	0.48 ± 0.73	0.85 ± 0.32	0.09 ± 0.10	0.04 ± 0.05
22:6n-3	3.82 ± 1.17	0.92 ± 1.04	0.60 ± 0.27	0.41 ± 0.37	0.13 ± 0.05
Σ n-3 PUFA	6.64 ± 1.71	3.87 ± 1.41	2.60 ± 0.68	2.17 ± 0.76	2.11 ± 0.41

Data are calculated as the percentage of identified fatty acids. Data shown are means ± SD of all six subjects taken at baseline on day 1 of the study. CE, cholesteryl ester; ND, not determined; PL, phospholipid; TG, triglyceride. *trans* analysis was not performed on the FFA fraction. A total 18:1t value was not obtained for CE.

[a] Only fatty acids of interest are shown; however, sums of subclasses include all fatty acids.

[b] The *trans*-fatty acid data were analyzed on a different column using blood samples from the elaidate tracer study only (see Materials and Methods).

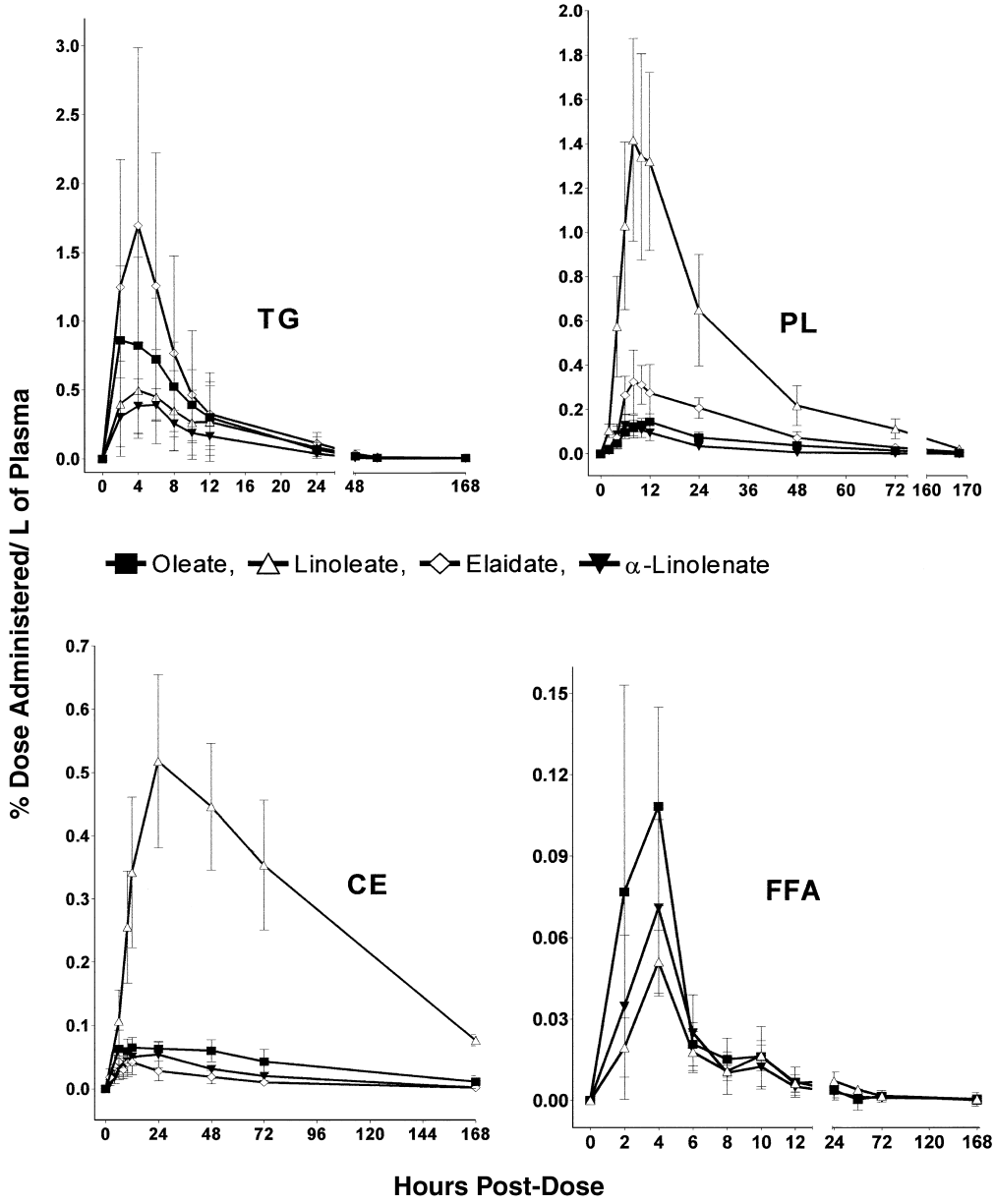

Fig. 2. Time course of ^{13}C enrichment data in plasma triglyceride (TG), phospholipid (PL), cholesteryl ester (CE), and FFA in six healthy women given [U-^{13}C]linoleate, (open triangles), [U-^{13}C]oleate (closed squares), [U-^{13}C]α-linolenate (closed inverted triangles), and [U-^{13}C]elaidate (open diamonds). Data are normalized for the concentration of fatty acids in plasma and the tracer dose administered. Data points indicate means ± SD. Note that the axes are scaled differently.

time of peak enrichment, tracer disappearance from plasma TG was exponential, with a half-life of 4–6 h, and there was no significant difference between tracers (**Table 4**). At 4 h postdose (which was close to peak enrichment in plasma TG), [^{13}C]linoleate in plasma TG and [^{13}C]linoleate oxidation rate were inversely related ($r = -0.94$, $P = 0.02$), as was [^{13}C]oleate oxidation compared with [^{13}C]oleate enrichment in the combined plasma lipid classes ($r = -0.94$, $P = 0.02$). Higher fasting plasma TG levels were associated with higher ^{13}C tracer in plasma TG at 4 h postdose for [^{13}C]linoleate ($r = 0.90$, $P = 0.03$), [^{13}C]oleate ($r = 0.93$, $P = 0.02$), and [^{13}C]elaidate ($r = 0.90$, $P = 0.02$).

In plasma PL, ^{13}C enrichment of all four tracers peaked at 6–10 h postdose and then followed a one-phase exponential decay pattern in all subjects ($r^2 = 0.93$–0.99; Fig. 2). Time to peak enrichment was significantly shorter for α-linolenate (6 h) than for the other three tracers (8–10 h; $P < 0.05$). Peak [^{13}C]linoleate incorporation into plasma PL was 5- to 10-fold that of the other three tracers (1.46 ± 0.46% dose/l plasma versus 0.15 ± 0.03 for [^{13}C]oleate, 0.34 ± 0.12 for [^{13}C]elaidate, and 0.13 ± 0.05 for [^{13}C]α-linolenate). The rank order of AUC for these tracers in plasma PL was [^{13}C]linoleate > [^{13}C]elaidate > [^{13}C]oleate > [^{13}C]α-linolenate ($P < 0.05$; Table 3). [^{13}C]Linoleate incorporation into plasma PL was 18-fold

TABLE 3. Area under the curve for plasma tracer incorporation

Fatty Acid	Area under the Curve				
	PL	CE	TG	Free Fatty Acid	Total Plasma (TG + PL + CE)
Linoleate	40.2[a] (31.9–46.4)	51.1[a] (38.9–59.2)	7.1[a] (4.8–8.7)	0.7[a] (0.6–0.8)	101.4[a] (80.7–114.9)
α-Linolenate	2.4[b] (2.0–2.6)	3.4[b] (3.2–3.8)	5.3[b] (3.4–6.7)	0.5[b] (0.4–0.6)	10.6[b] (9.1–11.6)
Oleate	5.1[c] (3.9–6.5)	6.5[c] (4.9–6.7)	9.5[c] (8.8–9.7)	0.6[a] (0.6–0.9)	19.6[c] (18.6–22.7)
Elaidate	11.9[d] (9.1–12.5)	1.8[b] (1.5–3.6)	13.1[d] (11.8–13.6)	—[a]	26.5[d] (20.2–32.0)

Values shown are medians, and values in parentheses are the 25th–75th percentiles. Values represent mg ^{13}C/l plasma/mg ^{13}C administered. Values with different numbered superscripts within each column are significantly different at $P < 0.05$ (Friedman repeated-measures ANOVA on ranks with Student-Newman-Keuls as the post hoc test).

[a]—, no data.

more than for [^{13}C]α-linolenate, 8-fold more than for [^{13}C]oleate, and 3.5-fold more than for [^{13}C]elaidate (Table 2). [^{13}C]Elaidate had the longest half-life in plasma PL and the slowest rate of decay (K), whereas [^{13}C]α-linolenate had the shortest half-life (Table 4). The AUC for [^{13}C]linoleate enrichment in plasma PL was negatively correlated with whole-body lean tissue volume ($r = -0.94$, $P = 0.02$).

In plasma CE, there was both slower enrichment and slower nonexponential decay of all of the tracers than in the other plasma lipid classes (Fig. 2). Far more [^{13}C]linoleate than the other three tracers was incorporated into plasma CE ($P < 0.05$; Table 3). Time to reach peak enrichment in plasma CE was significantly shorter for [^{13}C]elaidate than for the other three tracers (median of 6 h versus 12–24 h for the other tracers). There was an inverse correlation between [^{13}C]elaidate enrichment in CE and the percentage of elaidate in both plasma PL ($r = -1.00$, $P = 0.003$) and adipose tissue fatty acids ($r = -0.94$, $P = 0.02$). The AUC for [^{13}C]linoleate enrichment in plasma CE negatively correlated with both total ($r = -0.89$, $P = 0.03$) and subcutaneous ($r = -0.94$, $P = 0.02$) adipose tissue volume.

^{13}C enrichment in plasma FFA peaked at 2–4 h postdose, with peak values significantly higher for [^{13}C]oleate than for [^{13}C]linoleate or [^{13}C]α-linolenate (Fig. 2; no data were obtained for [^{13}C]elaidate in plasma FFA). Based on the AUC, [^{13}C]α-linolenate had the lowest enrichment in FFA ($P < 0.05$; Table 3).

When data from each of the three individual esterified plasma lipid classes studied were combined (TG + PL + CE), the total AUC for [^{13}C]linoleate was 4- to 10-fold higher than that for the other three tracers, with [^{13}C]elaidate > [^{13}C]oleate, followed by [^{13}C]α-linolenate ($P < 0.05$). The decay curves for ^{13}C in plasma total lipids were exponential, with [^{13}C]linoleate having an ~4-fold longer half-life (24 h) than the other three tracers (Table 4).

TABLE 4. Kinetics of tracer disappearance

Fatty Acid	PL	TG	Total Plasma (PL + TG + CE)
Linoleate	13.8[a] (12.7–14.7)	4.7 (4.5–5.1)	24.2[a] (19.4–28.8)
α-Linolenate	9.7[b] (7.3–12.3)	6.4 (5.6–6.9)	6.2[b] (5.8–10.3)
Oleate	16.6[a] (14.0–18.0)	4.9 (3.8–5.9)	7.1[b] (5.8–9.7)
Elaidate	19.1[c] (15.4–22.7)	4.0 (3.3–4.1)	5.9[b] (4.8–6.2)

Values shown are median half lives (h) with the 25th–75th percentiles shown in parentheses. Values with different lettered superscripts within each column are significantly different at $P < 0.05$ (Friedman repeated-measures ANOVA on ranks with Student-Newman-Keuls as the post hoc test). Exponential decay curves were determined on a per subject basis, and calculations were performed using a one-phase exponential decay model, where K is the disappearance rate constant and the half-life is $0.6932/K$. Clearance of tracer in CE and FFA lipid fractions did not fit an exponential decay pattern.

Tracer desaturation and chain elongation

Among the n-3 long-chain PUFAs derived from [^{13}C]α-linolenate, peak ^{13}C enrichments in plasma TG were highest for [^{13}C]eicosapentaenoate (0.05% dose/l plasma) and lowest for [^{13}C]docosahexaenoate (0.006% dose/l plasma; **Fig. 3**). ^{13}C enrichment values for the intermediate n-3 long-chain PUFAs, 20:3n-3 and 20:4n-3, could not be calculated because of low or undetected tracee concentrations. In plasma PL, peak ^{13}C enrichments ranged from 0.012% dose/l plasma for [^{13}C]eicosapentaenoate to 0.0028% dose/l plasma for [^{13}C]docosahexaenoate (Fig. 3). In plasma CE, the only ^{13}C-enriched long-chain PUFA derived from [^{13}C]α-linolenate that was detected was [^{13}C]eicosapentaenoate. When the n-3 long-chain PUFAs in all four plasma lipid classes were combined at each time point, the AUCs were 1.53, 0.56, and 0.34% dose/l plasma for [^{13}C]eicosapentaenoate, [^{13}C]docosapentaenoate (22:5n-3), and [^{13}C]docosahexaenoate, respectively. Summing the plasma lipid enrichments together, [^{13}C]eicosapentaenoate reached a plateau at 24–48 h postdose, [^{13}C]*n*-3-docosapentaenoate plateaued at 48–72 h postdose, and enrichment in [^{13}C]docosahexaenoate were still increasing at the last time point studied (168 h postdose), leading to a potential underestimation of [^{13}C]docosahexaenoate formation.

For n-6 long-chain PUFAs derived from [^{13}C]linoleate in plasma PL, ^{13}C enrichment was 0.010% dose/l plasma for [^{13}C]dihomo-γ-linolenate (20:3n-6) and 0.005 for [^{13}C]arachidonate (20:4n-6), which was either still increasing or plateauing at the last time point (168 h postdose; Fig. 3). In plasma CE in four of the six subjects, ^{13}C enrichment was detected in [^{13}C]γ-linolenic acid (18:3n-6), which first appeared at 10 h, and peaked at 24–48 h postdose. The mean peak ^{13}C enrichment (% dose/l plasma) was 0.004 for [^{13}C]γ-linolenic acid, 0.002 for [^{13}C]-dihomo-γ-linolenate, and 0.004 for [^{13}C]arachidonate (Fig. 3). AUC values (% dose/l plasma) for ^{13}C-enriched n-6 long-chain PUFAs derived from [^{13}C]linoleate were 0.33 for [^{13}C]γ-linolenic acid, 1.35 for [^{13}C]dihomo-γ-linolenate, and 0.94 for [^{13}C]arachidonate. Significant enrich-

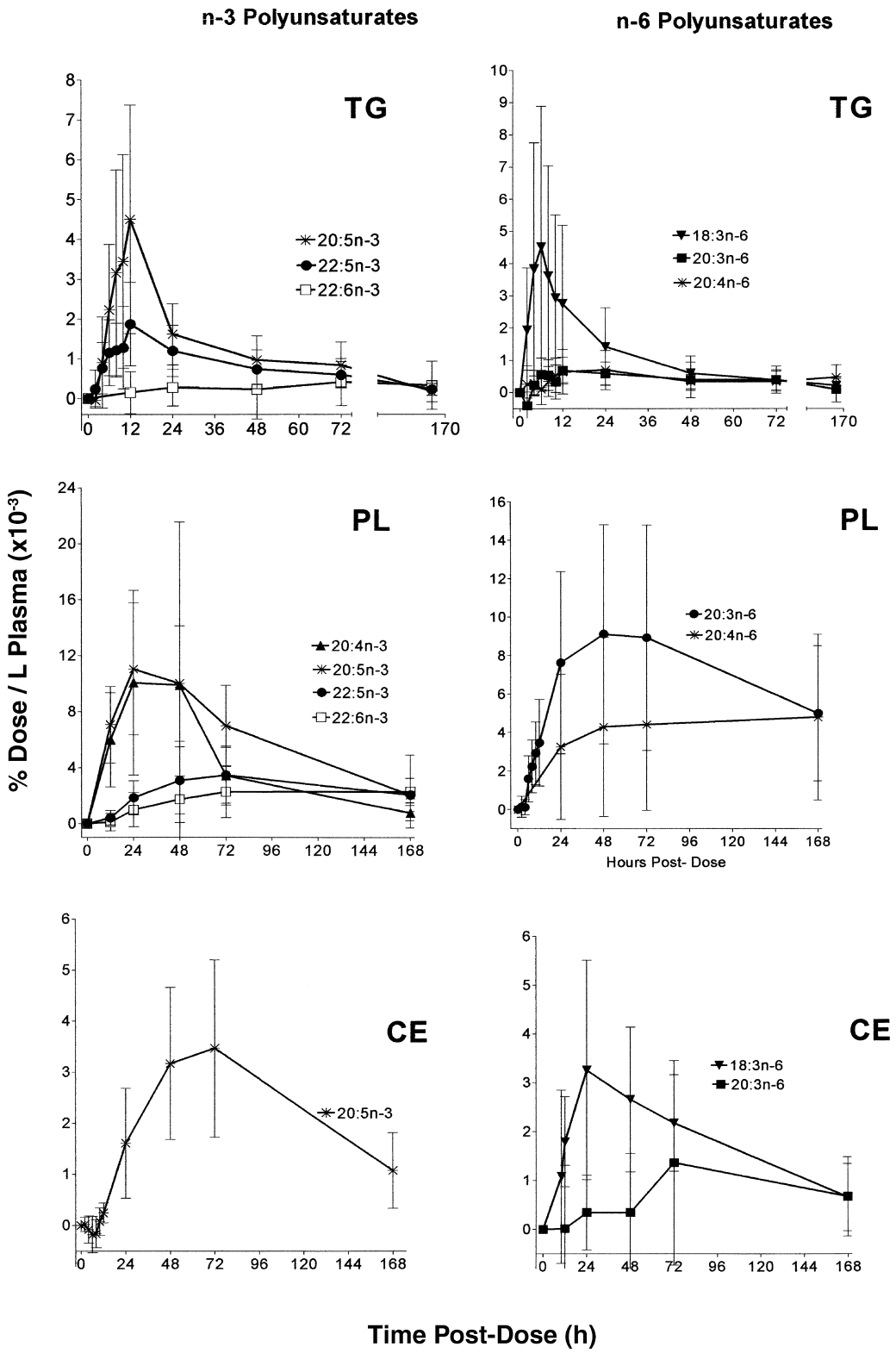

Fig. 3. Time course of ^{13}C enrichment in desaturation chain-elongation products in plasma TG, PL, and CE after an oral dose of [U-^{13}C]linoleate (left side) or [U-^{13}C]α-linolenate (right side) in six healthy women. Data points indicate means ± SD.

ment was observed for [^{13}C]eicosadienoate (20:2n-6). However, this peak coeluted with 20:3n-9, so quantitative enrichments could not be obtained. No ^{13}C was detected in adrenate (22:4n-6) or docosapentaenoate (22:5n-6) during the time course of this study.

Adipose tissue

^{13}C enrichment in adipose tissue was measured for each tracer except [^{13}C]oleate, for which the high oleate content in adipose tissue caused excess ^{13}C dilution (Table 3). Adipose tissue enrichment after dosing with [^{13}C]α-lino-

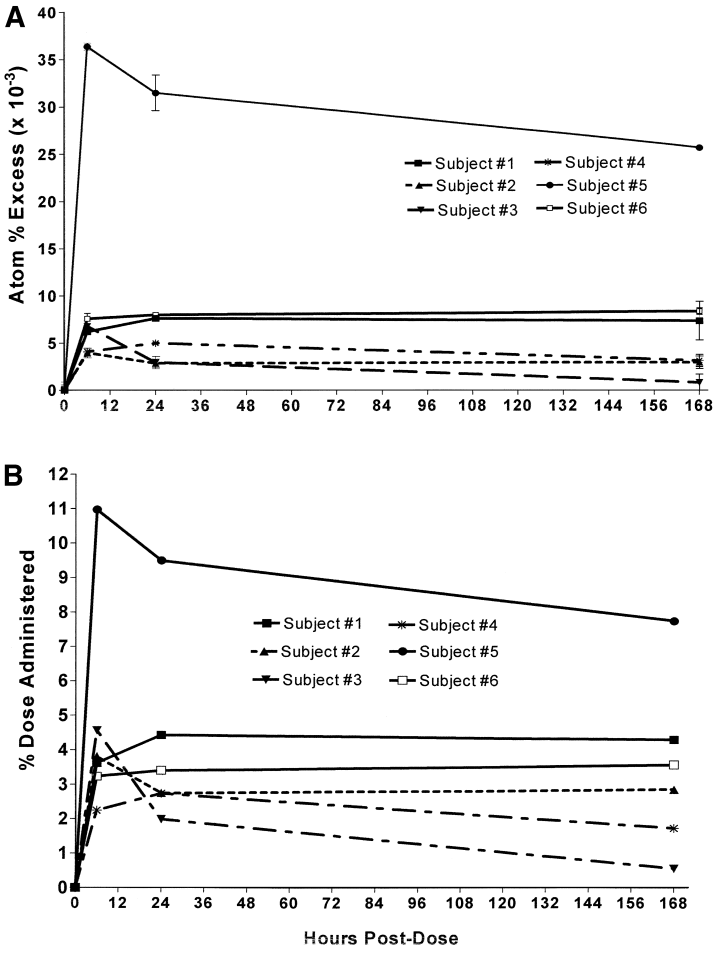

Fig. 4. Time course of ^{13}C enrichment in abdominal adipose tissue after an oral dose of [U-^{13}C]α-linolenate showing data for individual subjects. A: Mean atom percent excess at 6, 24, and 168 h after dosing. Data indicate means ± SD for two to four sample injections. B: ^{13}C enrichment data normalized for the concentration of fatty acids in adipose tissue and the amount of tracer administered.

lenate is reported in detail because it had the highest tracer-tracee ratio (**Fig. 4**). Maximal ^{13}C enrichment at 6 h postdose was seen in all subjects, after which it either plateaued (n = 4) or started to decrease (n = 2). Between 2% and 11% of the [^{13}C]α-linolenate was present as such in abdominal fat at 6 h postdose. Based on the calculated fat content of the whole body, this would have been 15–81% of the administered dose. At 24 h postdose, 2–9% of the [^{13}C]α-linolenate dose was in the abdominal fat (13–70% on a whole-body fat basis). By 168 h postdose, 0.6–8% of the [^{13}C]α-linolenate dose was in the abdominal region (or 4–57% on a whole-body fat basis). The whole-body fat calculations make the potentially inaccurate assumption that the tracer was taken up similarly in all adipose tissue regions.

[^{13}C]Elaidate enrichment in abdominal adipose tissue increased throughout the 168 h study period, averaging 4% of dose at 6 h, 6% at 24 h, and 8% at 168 h postdose. There was a trend toward greater ^{13}C enrichment at 168 h after [^{13}C]elaidate than after either [^{13}C]linoleate or [^{13}C]α-linolenate ($P = 0.11$), with no statistical difference at 6 or 24 h postdose (**Fig. 5**). At 6 h postdose, although adipose tissue ^{13}C enrichment was two to three times higher for [^{13}C]linoleate than for the other two tracers, these values were not significantly different. At 24 h postdose, adipose tissue ^{13}C enrichment differences between tracers were small (4, 6, and 6% of dose for [^{13}C]α-linolenate, [^{13}C]linoleate, and [^{13}C]elaidate, respectively). There was no significant relationship between abdominal adipose tissue volume and tracer enrichments in plasma or CO_2. However, at 24 h postdose, both total adipose tissue ($r = -1.00$, $P = 0.003$) and total subcutaneous adipose tissue volumes ($r = -0.94$, $P = 0.02$) correlated negatively with [^{13}C]elaidate enrichment in adipose tissue. [^{13}C]Elaidate enrichment in adipose tissue at 6 h postdose correlated negatively with cumulative [^{13}C]elaidate oxidation at 12 h postdose ($r = -0.89$, $P = 0.03$), which was not seen for [^{13}C]linoleate or [^{13}C]α-linolenate.

Figure 5 shows the whole-body distribution of ^{13}C as percentage of dose for all tracers (excluding oleate) in breath, total esterified plasma lipids, and abdominal adipose tissue. At both 6 and 24 h postdose, the plasma and adipose tissue compartments made up a higher proportion of whole-body enrichment for [^{13}C]elaidate and [^{13}C]linoleate than for [^{13}C]α-linolenate. Significantly lower [^{13}C]linoleate oxidation at 6 h was offset by its higher incorporation in both plasma and adipose tissue; however, these differences were not significant. By 168 h postdose, most of the ^{13}C from all three tracers had disappeared as $^{13}CO_2$ in breath, and little remained in plasma lipids.

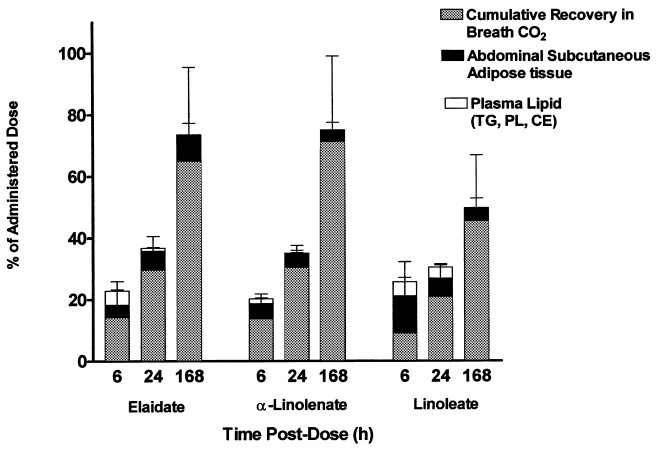

Fig. 5. Distribution of ^{13}C-labeled fatty acids between total plasma lipids, adipose tissue, and breath $^{13}CO_2$ at 6, 24, and 168 h after an oral dose of three fatty acid tracers. Data indicate means ± SD for six subjects.

DISCUSSION

This is the first description of the metabolism of labeled fatty acids that combines analysis of ^{13}C appearing in adipose tissue, plasma lipid fractions, and excretion in breath. Previously, several deuterated fatty acids were administered to compare incorporation into plasma lipid classes as well as the conversion of linoleate and α-linolenate to their desaturated and chain elongated products (17–20). Using ^{13}C or ^{14}C as the tracer, the simultaneous measurement of oxidation and plasma lipid enrichment of linoleate (21, 22) and α-linolenate (23–26) and their desaturated and elongated products was performed. However, in each case only a single fatty acid was studied. The analysis of all three compartments at once (plasma, adipose tissue, and breath CO_2) enhances the ability to understand the whole body utilization of these fatty acids. Our results show that after oral ingestion of these common fatty acids, adipose tissue is rapidly and extensively labeled. Under our experimental conditions, no major differences in abdominal subcutaneous adipose tissue uptake of linoleate, elaidate, or α-linolenate were discernible.

Our results concerning the quantitative recovery of ^{13}C in breath CO_2 during the first 24 h postdose and the rank order of oxidation of these four common dietary fatty acids agree with similar previously published results (21–33). Tracer studies reporting breath data do not often extend beyond 24 h, but in 22 of the 24 breath tracer profiles in the present study, ^{13}C enrichment in breath CO_2 was still above baseline at 168 h (8 days) postdose. In fact, as reported elsewhere (31–33), the period between 24 and 168 h postdose accounts for a significant proportion of the excretion on breath of common dietary fatty acids. Although much of this slow ^{13}C recovery in breath could be attributable to the retention of labeled CO_2 in the bicarbonate pool, the continued detectable enrichment in plasma and adipose tissue demonstrates that significant amounts of usable tracer fatty acid was still present in the body long after the first 24 h postdose. It is also important to note that some β-oxidized fatty acids may have been recycled to other fatty acids or cholesterol, such that the ^{13}C would not appear in breath CO_2.

The slower oxidation of linoleate compared with these other 18-carbon unsaturated fatty acids deserves comment. It is intermediate in pool size between oleate (higher) and α-linolenate or elaidate (both much lower), yet was oxidized more slowly than these other fatty acids (Fig. 1). Data from Bretillon et al. (30), who compared the oxidation of TG enriched with [1-^{13}C]α-linolenate and [1-^{13}C]linoleate as well as their *trans* counterparts, [1-^{13}C]9*cis*,12*trans*-linoleate and [1-^{13}C]9*cis*,12*cis*,15*trans*-α-linolenate, in healthy men, implicate a stereochemical effect in the slower oxidation of linoleate. The *trans* forms of α-linolenate were oxidized similarly to the all *cis* form; however, the *trans* and *cis* forms of linoleate were oxidized differently. Likewise, in the present study, oxidation of oleate and its *trans*-isomer elaidate were not different. Either the shape of linoleate seems to enhance its ability to be esterified or it reduces its affinity for the oxidative pathway. Emken et al. (34) showed that the incorporation of deuterated 12*cis*,15*trans*-linoleate into plasma CE and phosphatidylcholine was more similar to that of the saturated fatty acids and oleate than to that of all *cis*-linoleate. Therefore, unlike the other fatty acids, conversion of a *cis* to a *trans* double bond in linoleate dramatically affects its metabolism, indicating that its particular affinity for incorporation into plasma PL probably depends on its stereochemistry.

In the present study, the percentage of linoleate and α-linolenate in plasma PL fatty acids correlated positively with the oxidation of [^{13}C]elaidate, and linoleate was associated with its own oxidation. Because higher linoleate and α-linolenate in plasma PL is usually attributable to their higher dietary intake, diets rich in these two PUFAs would seem to increase oxidation and reduce adipose tissue retention of *trans*-fatty acids. Previously, consumption of a high versus low polyunsaturated:saturated 30% fat diet led to a higher oxidation of labeled palmitate and decanoate (35). In addition, postprandial fatty acid oxidation, as measured by indirect calorimetry, was higher on a high linoleic acid:saturated fat diet in eight normal-weight adults (36). As a potential mechanism, high PUFA diets are known to regulate gene transcription (37) by simultaneously inducing the transcription of genes involved in thermogenesis and fatty acid oxidation (e.g., mitochondrial uncoupling protein-3, carnitine palmitoyl transferase, acyl-CoA oxidase) while suppressing the transcription of lipogenic genes (e.g., acetyl-CoA carboxylase, fatty acid synthase). Our results suggest that a diet rich in linoleate or α-linolenate should promote more fatty acid oxidation and should help reduce the tissue accumulation of *trans*-fatty acids such as elaidate. A health consequence would be that the fatty acid composition of the rest of the diet may ameliorate some of the deleterious health effects of elaidate by increasing its removal in the postprandial period.

As shown previously (19, 20), a lower number of double bonds in the fatty acids we studied was inversely related to their abundance in plasma TG, i.e., oleate > linoleate > α-linolenate (Fig. 2, Table 3). Other experimental models, including human hepatoma cells (38), hepatocytes from neonatal and adult rats (39), and perfused rat liver (40), have also shown an inverse relationship between the amount of enrichment in TG and the degree of unsaturation of long-chain fatty acids. In terms of *cis/trans* conformation, elaidate is incorporated into TG to a greater extent than oleate when an intestinal cell line is used (41), whereas in a hepatic cell line, similar or lower incorporation occurs (42, 43), which may indicate differences in chylomicron incorporation. With these differences in TG fatty acid enrichment, the potential implication is that either high-linoleate or -α-linolenate diets would chronically induce lower fasting plasma TG levels than high-elaidate or -oleate diets. Also, in response to test meals with either high linoleate or α-linolenate, a lower postprandial TG response than seen in diets high in elaidate or oleate would be found (short term). Although feeding trials have examined both of these chronic and acute responses to dietary fatty acids, much of the results are inconclusive.

Our present results demonstrate that, compared with α-linolenate, oleate, or elaidate, linoleate is preferentially esterified to plasma PL and CE. This agrees with the higher proportion of linoleate than these other fatty acids that is normally found in plasma PL and CE (Table 2). Because plasma fatty acid profiles are a marker of tissue fatty acid profiles, greater acylation into plasma (and tissue) PL and CE could explain the greater whole-body retention rather than oxidation of linoleate. Whether this decreased oxidation of linoleate is attributable to its higher affinity for the glycerolipid pathway or its lower affinity for carnitine palmitoyl transferase, either of which would lead to less linoleate being available for oxidation, is unknown. Fatty acyl-CoAs are partitioned between either oxidation or glycerolipid (PL and TG) synthesis, with the latter being formed from a common substrate, diacylglycerol (44, 45). Therefore, the results from the present study indicate that linoleate, perhaps through a combination of being a poorer substrate for oxidation and being a better substrate for PL acyltransferases, is diverted toward the PL pathway, without particularly high incorporation into TG.

Previously, only two in vivo studies in humans, both using ^{14}C-fatty acids, have reported tracer enrichment in adipose tissue. Ormsby, Schnatz, and Williams (46) injected intravenous [1-^{14}C]linoleate bound to albumin into three men and measured ^{14}C enrichment in adipose tissue. The average enrichment was 9% of the dose at 10 min, 20% at 4.5 h, 10% at 10 h, and 11% at 24 h, values that are similar to ours (Fig. 5). Marin, Rebuffe-Scrive, and Bjorntorp (47) reported the preprandial and postprandial femoral and abdominal adipose tissue uptake of [U-^{14}C]oleate in 16 healthy premenopausal women. At 4 h postdose, 23% was incorporated into adipose tissue (average of both sites), up to 36% at 24 h, with a further slight increase up to 1 month postdose. Both of these studies demonstrate that uptake of fatty acids in human adipose tissue in the postprandial phase is rapid but that mobilization of tracer fatty acids from fat is relatively slow. Importantly, different adipose tissue regions may take up these fatty acids in different amounts; in addition to having a higher turnover, abdominal adipose tissue had ~15% higher uptake of tracer at 4 h postdose than did femoral adipose tissue (47). In the present study, the α-linolenate data were the most reliable, because its tissue pool size is fairly small, creating less dilution of the tracer and consequently higher [^{13}C]α-linolenate enrichments above baseline. On a whole-body basis, we observed similar time courses and percentages of incorporation of [^{13}C]α-linolenate (33% at 6 h, 29% at 24 h, and 24% at 168 h) as reported in these other two studies (46, 47).

The partitioning of dietary fat between oxidation and storage in adipose tissue potentially plays a role in the mechanism underlying hyperlipidemia and obesity. In support of this, hyperlipidemic men had four times more [^{14}C]linoleate in TG and also oxidized 30% less of the tracer than normolipidemic men (21). Similarly, in this study for three of four tracers, there was an association between fasting TG and tracer presence in TG. In terms of obesity, adipose tissue postprandially clears and stores more fat from nondiabetic obese women than from lean females, accompanied by a higher postprandial insulin and an earlier acylation-stimulating protein response (48).

In summary, under our study conditions, the major route of utilization of all four of the tracer fatty acids was oxidation, followed by storage in adipose tissue. Breath CO_2 accounted for between 46% and 71% of the administered dose. Abdominal adipose tissue accumulated between 5% and 12% of dose administered, which translates to ~30–70% on a whole-body adipose tissue basis. Maximal incorporation of tracer into plasma ranged from 3% to 8% depending on the tracer administered. The ω3 and ω6 long-chain PUFAs in plasma had a maximum value of less than 0.1% of the administered dose, values that agree with other recent data (22–26, 49). These net desaturation chain-elongation values are derived from the plasma pool but should at least qualitatively mirror the tissue incorporation of these fatty acids and their long-chain products.

Beynan and Katan (50) have previously suggested that fatty acids that are retained rather than oxidized have a hyperlipidemic effect, attributable to being available for VLDL output by the liver, that consequently may result in decreased plasma LDL. This hypothesis results primarily from a comparison of the metabolism of saturated and unsaturated fatty acids in the literature. In the present study, elaidate, which has not been studied as extensively as some of the saturated fatty acids, particularly 16:0, was studied rather than a saturated fatty acid as a "hyperlipidemic" fatty acid. The results in the present study indicate that this hypothesis is perhaps a simplistic view. Linoleic acid, which has long been known to have potent blood cholesterol-decreasing effects and HDL-cholesterol-increasing effects, was less oxidized, particularly in the postprandial period, and was retained more in plasma lipid than the other three tracers. Conversely, higher linoleic acid in

plasma PL was associated with greater oxidation of fatty acids. However, the strength of the present study is the examination of tracer incorporation into the other plasma lipid fractions. Linoleic acid was markedly incorporated into plasma PL, which may be a marker of tissue membrane uptake and subsequently into CE. However, compared with the other fatty acids, it was not incorporated to a great extent into plasma TG. Elaidate, on the other hand, which was oxidized similarly to oleate and α-linolenate, had the highest incorporation into plasma TG. Also, it was esterified quite poorly to cholesterol, which may be a factor in reverse cholesterol transport and in elaidate's HDL-cholesterol-decreasing effect.

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